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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Attorney Docket No. 071007/0137

In re application of:
BHATTACHARJEE *et al.*

Group Art Unit: 1641

Serial No.: 08/886,044

Examiner: S. Devi

Filing Date: June 30, 1997

For: *VACCINE AGAINST GRAM-NEGATIVE BACTERIAL INFECTIONS*

ENTERED

AMENDMENT AND REQUEST FOR RECONSIDERATION UNDER 37 CFR §1.116

Assistant Commissioner of Patents
Washington, D.C.

Sir:

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In reply to the Official Action dated August 19, 1999, applicants request that the PTO cancel claims 19 and 20, without prejudice or disclaimer. With this cancellation, claims 1-3, 5-8, and 12-17 are pending and stand rejected.

Initially, applicants note that cancelled claims 19 and 20 were rejected under the second paragraph of Section 112, and that claim 19 was rejected separately, under the first paragraph of Section 112. In addition, the examiner had rejected claims 19 and 20 under Section 103(a), based on Ziegler *et al.* (1973) in view of Zollinger *et al.* (U.S. 4,707,543) and Ziegler *et al.* (1982). These issues have been dealt with in Serial No. 08/467,047. Accordingly, claims 19 and 20 have been canceled, mooting the pending double patenting rejection.

The examiner has maintained an obviousness rejection of claims 1-3, 5-8 and 15-17 over Zollinger *et al.* (U.S. 4,707,543) in view of Ziegler *et al.* (1982) or Myers *et al.* and Munford *et al.* The examiner urges that Zollinger discloses the use of capsular polysaccharide or lipopolysaccharide to solubilize an outer membrane protein. The examiner admits that

Zollinger does not teach J5 *E. coli* polysaccharide complexed with meningococcal outer membrane protein, but urges that one of ordinary skill in the art would have been motivated to use LPS from the J5 strain instead of LPS from other strains "as an immunogen to treat sepsis caused by multiple gram negative bacterial pathogens." As further support for the substitution, the examiner notes that Myers teaches that "the core region is highly conserved among LPSs obtained from different genera of *Enterobacteriaceae*" and that *E. coli* J5 has "a partially complete (and therefore antigenically cross-reactive) core region."

In the current action, the examiner provides a very thorough review of the literature relevant to E. *coli* J5 vaccines. From the examiner's perspective, informed by this review, one certainly would expect that an effective endotoxin vaccine was approved long ago. This is especially so when one considers that nearly four hundred thousand cases of sepsis a year are documented, and that sepsis is the leading cause of death in intensive care units.

Clearly, then, there is a long-felt need and a substantial commercial market for such a vaccine, the search for which has marked a major involvement of modern biotechnology in clinical medicine. Yet, nearly a quarter of a century after the first publications cited by Examiner Devi, there still is no vaccine to prevent or alleviate the severity of sepsis. This fact highlights the sharp contradiction between the aforementioned perspective and the reality of contemporary endotoxin-vaccine development.

The primary reference, Zollinger, describes a process for preparing detoxified polysaccharide-outer membrane protein complexes. The polysaccharide may be capsular polysaccharide or detoxified lipopolysaccharide, although only capsular polysaccharides are exemplified. Furthermore, complexes with lipopolysaccharides are prepared in Example 3 of the Zollinger patent but never are tested for bactericidal antibody response. Rather, the ***purpose*** attributed by Zollinger to the polysaccharide, whether capsular polysaccharide or lipopolysaccharide, relates to solubilizing the outer membrane proteins. Thus, Zollinger speaks of "outer membrane proteins...solubilized by the tetravalent mixture of A, C, Y, and W135 polysaccharides" (column 2, lines 7-9), and states that "the detoxified [lipopolysaccharide] was shown to retain its ability to bind to and ***solubilize*** outer membrane proteins" (column 8, lines 66-68); also, "sonication is often essential to facilitate the protein-

lipopolysaccharide interaction and ***solubilize*** the protein" (column 9, lines 13-15). For the purpose of solubilization, Zollinger informs the artisan of ordinary skill that either detoxified lipopolysaccharide or capsular polysaccharide is suitable.

The examiner has combined Zollinger with Ziegler *et al.*, a study of passive immunization with antiserum from animals immunized with whole bacteria. In their landmark study, Ziegler demonstrated that a vaccine made from J5 *E. coli* could induce an antiserum that prevented mortality from septic shock.

In the Ziegler study, however, the researchers were unable to identify antibodies as a basis for that protection. Thus, Zanetti *et al.* (1991) state that, "as was noted in the report by Ziegler *et al.*, protection was related to immune plasma, not to specific levels of antibody to core LPS in a given plasma" (first paragraph on page 988), and, "as already noted, in both successful clinical studies with *E. coli* J5 antiserum, the protection remained of unclear origin because outcome could not be convincingly correlated with the level of antibodies to the core LPS of *E. coli* J5...the protection afforded by *E. coli* J5 antiserum could not be attributable to antibodies to the LPS of *E. coli* J5" (second full paragraph on page 988). Similarly, Glauser *et al.* note that "a favorable outcome could not be correlated with antibody titers in either of the two clinical studies done with human polyclonal antisera to J5...the mechanisms of protection by antisera to J5 remain unknown" (second full paragraph on page S208). Baumgartner likewise states that "the successful studies did not discover the factor responsible for the postulated crossprotection in J5 antiserum, because the protection could not be attributed to anti-J5 LPS, anti-Re LPS, or anti-lipid A antibodies" (top of page 923). Further, Greisman *et al.* cite data that implicate factors in J5 and R595 antisera, other than antibodies to J5 or R595 LPS core epitopes or lipid A, which mediate the reputedly broad-spectrum protection of such antisera.

The examiner has not properly cast the applicable state of the art in light of contemporaneous progress in the relevant scientific field. For example, the studies of Myers and Munford are cited, on page 3 of the action, as if they represented some sort of eternal truth. Yet the hypothesis advanced in these documents, that the core structures of LPS are very similar, has been contradicted by more recent data, conclusively showing that there is

considerable heterogeneity in the LPS core. For example, see applicants' discussion, in their previous response, regarding the production by Lugowski of four different conjugate vaccines from different core structures of *E. coli*. Similarly, DiPadova and colleagues generated their core LPS-specific monoclonal antibody by sequential immunization of animals with different LPS core structures. Even when animals were immunized with a variety of LPS core structures, however, the resulting monoclonal antibody had no activity against *Klebsiella* or *Pseudomonas*.

In the present case, a "picking and choosing" approach to the various published studies has given the examiner an inaccurate impression of what would have been obvious in the field of endotoxin vaccines. In fact, no reasonable permutation of teachings from the prior art provides the basis for extrapolating to a successful vaccine against sepsis.

For instance, Examiner Devi's thinking seems heavily influenced by an uncited paper by Dunn and colleagues (1984), showing that immunization with whole bacterial cells or LPS induces cross-reactive antibodies to endotoxin (see Office Action dated August 19, 1999, at page 3). In that paper, however, a single horse was "hyperimmunized" with both heat killed bacteria and LPS, *i.e.,* it received multiple intravenous immunizations over a prolonged period of time amounting to several months, the exact regimen was not specified. Dunn demonstrates that antibodies to LPS could be induced and that IgG, following J5 immunization, provided heterologous protection when given passively. But such a hyperimmunization regimen would never be approved and in fact is infeasible for clinical practice.

Dunn's results in no way would have suggested how to make a vaccine with sufficient immungenicity that such hyperimmunization was not necessary. A practical vaccine must be immunogenic when given in a few doses, over a much shorter period of time, rather than intravenously, as in Dunn. This likely explains why Dunn and colleagues have never produced a vaccine for human use, based on their studies, even though they have had 15 years in which to do so.

Similarly, Examiner Devi cites studies by Moore and colleagues (1987) for showing that whole bacterial immunization with *E. coli* J5 or with native J5 LPS prevents graft-versus-host disease in mice. But a personal communication to Dr. Cross from Dr. Cohen, the senior

author of that study, indicated that Dr. Cohen never pursued this finding because he considered the approach in question, using whole bacterial vaccine or native LPS, as inadequate.

Examiner Devi also mentions the studies of Tomita *et al.* to demonstrate that a detoxified LPS vaccine would have been obvious. Indeed, these investigators showed that detoxification of LPS yields an immunogenic preparation. But these investigators never determined the functional activity of their antibody or its ability to bind to heterologous bacteria.

Taken together, the studies of Dunn, Moore, and Tomita indicate that detoxified LPS will induce antibodies (Tomita) and that immunization with a vaccine will have some functional activity in certain animal models (Dunn, Moore), but that the vaccines are inadequate immunologically or require excessive immunizations. The references lack the teaching that is necessary in order to produce a clinically useful vaccine.

The examiner has cited numerous other documents to illustrate the state of the relevant art. Because none of these documents is cited in a rationale for rejection, applicants will not discuss them in any detail. Suffice it to say that no proper combination of these documents would have substantiated a reasonable *a priori* expectation that detoxified LPS endotoxin from *E. coli* J5 strain could be employed with a purified *N. meningitidis* OMP, in a vaccine as claimed, to achieve results of protective immunogencity never previously attained.

Furthermore, applicants made the further, surprising discovery that, in patients acutely traumatized, there is a normal immune responsiveness to active vaccination. See Campbell *et al.*, *Clinical Infectious Diseases* 23:179 (1996). Also, applicants have submitted a manuscript documenting that during administration of a chemotherapeutic agent, cyclophosphamide, the antibody levels persist throughout the period of neutropenia. Recent studies, documenting a Th 2 polarization with increased production of two cytokines, IL-4 and IL-10, which are critical to the antibody response, provides a theoretical underpinning for applicants' discovery. By contrast, there is no teaching in the art that the immunocompromised target population for any endotoxin vaccine could be protected from sepsis by immunization. Indeed, the

perception in the art that such a population could **not** be immunized effectively is a compelling reason why applicants' therapeutic and prophylactic approach would not have been obvious.

Inexplicably, the examiner has given short shrift to applicants' most recent Rule 132 declaration. This declaration documented studies of challenge with virulent strains of heterologous bacteria following active immunization with J5 LPS/OMP. As described in the protocol appended to the declaration, rats rendered neutropenic with cyclophosphamide were immunized, either with de-O-acylated J5 LPS ("dLPS") complexed to OMP or with saline, in a 3-dose regimen prior to challenge with the heterologous bacteria. After immunization, the rats were challenged with either *Pseudomonas aeruginosa* or *Klebsiella pneumoniae*, in a dose that exceeded LD_{90} for this experimental model in previous studies.

The results showed that active immunization with J5 dLPS/OMP vaccine produced a prompt and sustained anti-core glycolipid antibody level that was generally in 100-fold excess of pre-immunization baseline levels. Twenty-four hours after bacteremia, antibody levels decreased, but then rapidly recovered to, and remained at, pre-infection levels. Active immunization with J5 LPS/OMP vaccine induced greater than 800 ELISA units/ml of antibody at the onset of neutropenia, nearly 4 weeks after the last dose of vaccine, and this level persisted throughout the entire period of neutropenia, for up to 80 days after the initial immunization. This is in distinct contrast to results achieved by passive immunization with antibodies, where initial levels of 800 ELISA units/ml of antibody dropped to less than 200 ELISA units/ml of antibody by 24 hours.

While immunization did not prevent either systemic infection or initiation of sepsis, it clearly reduced the likelihood of a lethal outcome following infections with both heterologous strains of bacteria. Vaccinated animals challenged with *Pseudomonas* had an overall survival rate of 48% compared to 7% for saline treated control animals. A similar result ensued with *Klebsiella* challenge, with a 64% survival rate for vaccinated animals versus a 13% survival rate for control animals.

Yet, in an Official Action that is twenty-one pages long, the examiner devotes only six sentences to the issue of applicants' declaration. On page 10 of the action, the examiner comments that

> Applicants submit a further declaration from Dr. Cross which provides results of challenge studies with heterologous bacteria. While the information in the declaration supports that provided in the specification, it does not overcome the rejection of instant claims under 35 U.S.C. 103(a).

There is no immediate substantiation provided for this statement, but much later, in the paragraph bridging pages 14 and 15 of the Action, the examiner explains that

> The data provided with Dr. Cross's declaration shows that administration of a vaccine-derived antiserum to animals results in 48% survival of animals against challenge with *Pseudomonas* and 64% survival against challenge with *Klebsiella*. However, 52% and 36% of immunized or treated animals respectively were not "protected." The full scope of the claims is not commensurate with the scope of the enabling disclosure and undue experimentation would be required by one of ordinary skill in the art to reproducibly practice the invention as claimed. The enablement (scope) provisions of 35 U.S.C. §112, first paragraph, are not met and the claim is viewed as non-enabled with respect to its scope.

Raised in connection with the declaration, this issue of scope is elaborated nowhere else in the action. The only "scope" rejection relates to claim 19, which was directed to passive, not active, protection. Accordingly, applicants do not understand the examiner's reference to "scope" at this point in the action.

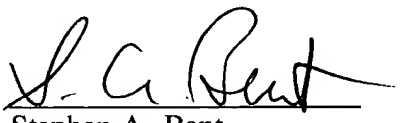
Vaccine claim 1 recites "a vaccine, effective in actively immunizing a subject against infection by heterologous Gram-negative bacteria or against lipopolysaccharide (LPS) endotoxin-mediated pathology by the production of an antibody, comprising a non-covalent complex between (i) purified, detoxified LPS endotoxin derived from *E. coli* J5 strain and (ii) a purified outer membrane protein (OMP) derived from *N. meningitidis*." It is difficult to see why this claim would be deemed not enabled. The claim specifically recites both the immunogen and the immunocarrier, as well as the fact that they form a non-covalent complex. The specification clearly describes how to make and use this vaccine, and the positive results documented in Dr. Cross's declaration derive from use of this same vaccine. Should the

examiner persist in such statements, applicants submit that he provide greater explication of their foundation.

The examiner's comments regarding the percentage of subjects that were protected, 48% and 64%, respectively, likewise are not understood. There are other vaccines in common use that provide significantly less than 100% protection. For example, pneumococcal immunization is routinely used, and its efficacy is generally believed to be between 60 and 70% at best. A similar degree of efficacy is the case with influenza immunization. Clearly, vaccines that provide a percentage of protection similar to that demonstrated for the present vaccine are considered to have clinical value. Applicants submit that the data in the second Cross declaration are in line with those for vaccines generally, and are sufficient to rebut any allegation with respect to the enablement or obviousness of the present invention.

In view of the foregoing amendments and remarks, it is believed that all claims are in condition for allowance. Reconsideration of all rejections and a notice of allowance are respectfully requested. Should there be any questions regarding this application, the examiner is invited to contact the undersigned attorney at the phone number listed below.

Respectfully submitted,



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Date

Stephen A. Bent
Reg. No. 29,768

FOLEY & LARDNER
Suite 500, 3000 K Street, N.W.
Washington, D.C. 20007-5109
USA
(202) 672-5300